Jurnal Eduhealth
Volume 15 , Number 02, 2024, DOI 10.54209/eduhealth.v15i02
ESSN 2808-4608 (Online)
https://ejournal.seaninstitute.or.id/index.php/healt

Antioxidant Activity Of Effervescent Granule Of Red Dragon Fruit Peel Extract (Hylocereus Polyrhizus W.) Using The DPPH Method

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Article Info
Keywords:
Hylocereus polyrhizus,
effervescent granules,
antioxidant.

ABSTRACT
Red Dragon Fruit Peel *(Hylocereus polyrhizus* W.*)* contains chemicals such as alkaloids, flavonoids, saponins, phenols, and steroids. Flavonoids and phenols are compounds that have antioxidant properties. Red Dragon Fruit Peel extract is formulated in effervescent granule dosage form with different concentrations of 0%, 20%, 25%, and 30% based on its antioxidant activity. The purpose of this study was to determine the effect of concentration differences on the antioxidant activity of effervescent granules produced. The research method uses a completely randomized design (CRD) which includes sampling, sample processing, phytochemical testing, extract preparation, effervescent granule preparation, effervescent granule physical quality testing, effervescent granule testing, antioxidant activity testing, and data analysis. Data from physical quality testing and the IC_{50} value of effervescent granules were analyzed descriptively using the Analysis of Variance (ANOVA) method with a 95% confidence level. The results obtained show that Red Dragon Fruit Peel extract can be formulated into effervescent granules, and the physical quality test results meet the physical quality requirements for all concentrations. The effervescent granule formulations produced at all concentrations have a very strong antioxidant effect (<50 ppm). The variation of Red Dragon Fruit Peel extract concentration in effervescent granule formulation has a significant effect on its antioxidant activity.

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INTRODUCTION

Free radicals are molecules with unpaired electrons in their outer orbit. Therefore, free radicals become highly reactive when they bind with surrounding electron molecules in search of a partner. Free radicals can come from biological activities inside and outside the body, especially in the form of air pollution, drugs, toxic substances, radiation, ultraviolet light, and others. Free radical compounds can cause oxidative reactions in the body. Under normal circumstances, oxidative reactions help fight inflammation, kill pathogens, and detoxify toxins. Excessive oxidative reactions can cause cell damage, reduce cell adaptation, cause cell death, and lead to disability and disease. The effects of free radicals on the body are difficult to detect, but they cause slow and serious effects. The adverse effects of free radicals can be neutralized with antioxidants (Abirami et al., 2021)

Antioxidants are natural substances or compounds that can protect body cells from free radical damage and aging. The human body can neutralize free radicals through endogenous

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antioxidants. However, if the amount of free radicals is too much, the ability to neutralize free radicals will decrease. The body needs exogenous antioxidants, which help protect the body from free radical attack by reducing their harmful effects (Gustaman et al., 2022). Exogenous antioxidants are obtained through the consumption of natural foods and beverages that are rich in antioxidant amino acids, vitamins, minerals, and bioactive compounds. Nutraceuticals are one of the products derived from exogenous antioxidant formulations. One of the fruits known to contain antioxidants is the Red Dragon Fruit Peel (Made Udayani Dwi Yadnya & Agung Gede Rai Yadnya Putra, 2022).

Red Dragon Fruit Peel is proven to be rich in antioxidants. According to (Manihuruk et al., 2017) the flesh of *Hylocereus polyrhizus W.* contains active components that can bind free radicals and is said to be a source of antioxidants. The main antioxidant compounds found in dragon fruit are polyphenols, vitamin C, and vitamin E (Made Udayani Dwi Yadnya & Agung Gede Rai Yadnya Putra, 2022). Dragon fruit skin also contains antioxidant compounds (Syaputri et al., 2023) states that the phenolic content and antioxidant activity of white dragon fruit and *Hylocereus polyrhizus W.* skin are higher than the fruit flesh. Dragon fruit is widely utilized by the community, but in reality, only the flesh and skin are commonly utilized. Dragon fruit is thrown away as waste. In this study, researchers used a sample of Hylocereus polyrhizus W. skin and utilized its antioxidant content by formulating effervescent granules from Hylocereus polyrhizus W. skin extract.

Effervescent granules are coarse granules or powders containing medicinal ingredients in a dry mixture. These preparations usually consist of sodium carbonate, carbonic acid, and tartaric acid. When water is added, the acid and carbonate react, releasing carbon dioxide and creating bubbles (Syaputri et al., 2023). Effervescent granules are an attractive alternative for the development of soft drink products, offering variety to the presentation of traditional drinks. The advantages of effervescent granules over ordinary powdered beverages are that they can produce carbon dioxide gas, provide a fresh taste, mask the bitter taste of medicinal ingredients, increase the absorption of medicinal ingredients in the stomach, and are convenient to store. This will happen. The transportation method is compared with liquid beverages (Pantria Saputri et al., 2020).

Antioxidant activity is tested using various methods, including β-carotene bleaching, also known as carotene bleaching, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical reduction, and thiobarbituric acid assay. In this study, the DPPH method was used to reduce free radicals. This is an assay that determines the antioxidant activity of the tested sample by examining its ability to resist DPPH free radicals. The principle of this test is that hydrogen atoms from the tested sample are released into DPPH radicals to form a non-radical compound diphenylpicrylhydrazine which is characterized by a change in color. This method is the simplest, fastest, and most cost-effective way to measure the ability of antioxidants in foods, fruits, and vegetables to reduce free radicals (Abirami et al., 2021).

Based on the above, this study was conducted to investigate whether red dragon fruit peel extract can be made into effervescent granule preparations, whether the effervescent granules produced have antioxidant activity, and whether changes in concentration have an effect or not. The antioxidant effect of red dragon fruit peel extract effervescent granules. The

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purpose of this study was to formulate effervescent granules from red dragon fruit peel extract and test the antioxidant activity of effervescent granules made using the DPPH free radical capture method.

METHODS

Materials

The tools used in this study are glassware (Scott), sieve mesh 20 and 40, stirring rod, maceration vessel, blender (Cosmos), porcelain cup, cuvette, mortar and stamper, oven blower, water bath (Memmert), micropipette (Biopette), drop pipette, drip plate, tube rack, horn spoon, UV-Vis spectrophotometer (Unico 2800 UV-VIS), stopwatch, test tube (Pyrex), analytical balance (Citizen). The materials used were citric acid (Brataco), citric acid (Brataco), distilled water, dextrin (Brataco), DPPH (1,1-diphenyl-2-picrylhydrazil), absolute ethanol (Merck), red dragon fruit skin (Hylocereus polyrhizus), mannitol (Merck), Na CMC (Brataco), sodium bicarbonate (Brataco), Dragendorff reagent, Lieberman Buchard reagent, Meyer reagent, Wagner reagent, polyvinylpyrrolidone (Merck).

Simplisia Preparation and Extraction Process

Red dragon fruit peel was washed thoroughly, then the skin was separated from the pulp. After that, the skin was cut and dried in a blower oven at 60^{0}C for 24 hours. The dried dragon fruit skin was pulverized using a blender and sieved using a 40 mesh sieve to obtain red dragon fruit skin powder. A total of 500 mg of red dragon fruit peel powder was extracted by maceration method, namely soaking the red dragon fruit peel powder in a mixture of solvents, ethanol, and distilled water in a 1:1 ratio in a tightly closed vessel and left for 15 minutes. The obtained macerate was separated using filter paper and the maceration process was repeated using the same solvent. The resulting macerate was collected and 15% dextrin was added. The extract that has been mixed with dextrin is then dried using a blower oven at 60^{0}C for 4 x 24 hours. The dried extract obtained was then pulverized and sieved with a 40-mesh sieve (Astika Winahyu et al., 2019).

Effervescent Granule Formulation of Red Dragon Fruit Peel Extract (*Hylocereus polyrhizus* W).

Table 1 Red dragon fruit peel extract effervescent granule formula

Material Name	Function	Formula (%)			
		F1	F2	F3	F4
Dried extract of red dragon fruit peel	Active substance	-	20	25	30
Sodium bicarbonate	Basic component	25	25	25	25
Citric acid	Acid component	11	11	11	11
Tartric acid	Acid component	14	14	14	14
PVP	Binder	2	2	2	2
Na.CMC	Suspending agent	1	1	1	1
Mannitol	Sweeteners and fillers	47	27	22	17

Description:
F1 : Effervescent granule formula without extract
F2 : Dragon fruit peel extract effervescent granule formula with 20% concentration.

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F3 : Dragon fruit peel extract effervescent granule formula with 25% concentration.
F4 : Dragon fruit peel extract effervescent granule formula 30% concentration

Manufacture of effervescent granules using the wet granulation method. This method uses a separate granulation process between the acid component and the base component. The granulation of the base component was carried out by drying sodium bicarbonate at 35-40^{0}C for 24 hours, then adding a dry extract of red dragon fruit peel, mannitol, Na CMC, and some PVP that had been dissolved in alcohol stir until smooth, then granulated with a mesh 20 sieve. Granulation of the acid component was carried out by drying citric acid and tartric acid at 35-40^{0}C for 24 hours, then adding the dried extract of red dragon fruit peel, mannitol, Na CMC, and the remaining PVP stirred until smooth, then sifted using a mesh 20 sieve. The granules of each acid and base component were dried in an oven at 60^{0}C for 15 minutes. After drying, the two components were mixed and then sieved again with a 40-mesh sieve (Made Ayu Nila Septianingrum et al., n.d.)

Physical Quality Testing of Effervescent Granule of Red Dragon Fruit Peel Extract *(Hylocereus polyrhizus* W*).*

Organoleptic test

Organoleptic tests include odor, color, and taste tests. Odor testing is done by placing the effervescent granule on the palm and smelling the aroma. Color is tested by direct observation of the color of the effervescent granule produced. Taste testing is done by taking a little effervescent granule then placed on the tip of the tongue and tasted for about 10 seconds. The odor, color, and race produced are as similar as possible to one another.

Moisture content

4 g effervescent granule was dried in an oven at 105-110^{0}C for 3 hours. The difference in weight before and after drying is the amount of water evaporated. Moisture content was calculated by the following formula:

$$\text{Moisture content} = \frac{\text{Wet Granule Weight} - \text{Dry Granule Weight}}{\text{Wet Granule Weight}} \times 100\%$$

Flow rate and angle of repose

The working procedure to obtain granules with good quality is that 4 g of effervescent granules are put into a funnel with the bottom closed. Open it slowly until all the granules come out of the funnel and form a pile on a flat surface. The time it takes for the granule to start falling out of the funnel to form a pile is the flow velocity. The angle of repose can be calculated by the following formula:

$\text{Tan } \alpha = \frac{H}{r}$

Description:

α = angle of repose
h = cone height (cm)
r = radius of the cone (cm) (Hasibuan & Sumartini, 2020)

Dissolving time

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The test method is to put 4 g of effervescent granule of each formula into 100 ml of distilled water. The dissolving time is calculated by using a stopwatch starting from the granule immersed in distilled water until all the granules are dissolved and the bubbles around the container begin to disappear. The dissolving time of a good effervescent granule ranges from 1-2 minutes. An effervescent granule is said to meet the requirements of the water content of the preparation if the effervescent granule is well dispersed in water within 1-2 minutes (Hasibuan & Sumartini, 2020).

Antioxidant Activity Testing of Effervescent Granule of Red Dragon Fruit Peel Extract (*Hylocereus polyrhizus* W).

Preparation of DPPH Solution and Test Sample Preparation

Dissolve 50 mg DPPH in 100 ml absolute ethanol (50 ppm concentration DPPH solution), then weigh the effervescent granule of red dragon fruit peel extract from each formula as much as 0.5 g each. then dissolve the granule with absolute ethanol up to 25 ml in a volumetric flask. Then filtered and collected the filtrate (concentration of 20,000 ppm), this is the standard solution. Take 0.01 ml of the standard liquor and suffice the volume with absolute ethanol in a 10 ml volumetric flask (to obtain a concentration of 20 ppm). Each 20 ppm concentration solution was pipetted as much as 0.5 ml; 2.5 ml; 5 ml; and 7.5 ml, then entered into a 10 ml volumetric flask and sufficed the volume with absolute ethanol (to obtain test solutions with concentrations of 1 ppm, 5 ppm, 10 ppm and 15 ppm) (Syaputri et al., 2023).

Preparation of Test Sample Solution and Blank Absorbance Measurement

Each test solution was pipetted as much as 1 ml, DPPH solution as much as 1 ml and absolute ethanol as much as 2 ml in a measuring flask and shaken. Then the test sample solution of each concentration was stored in a dark room for 30 minutes. 50 ppm DPPH solution was pipetted as much as 5 ml and stored for 30 minutes in a dark room, then measured the absorption with a UV-Vis spectrophotometer at a wavelength of 515 nm. All work was carried out in a closed room and avoided sunlight (Artikel et al., 2016).

Measurement of Antioxidant Activity of Test Samples

The test solution that has been made and kept for 30 minutes is measured by a UV-Vis Spectrophotometer at a wavelength of 515 nm. DPPH radical-capturing activity (%) was calculated by the following formula:

$$\% \text{ inhibition} = \frac{\text{A Blanko} - \text{A Sample}}{\text{A Blanko}} \times 100\%$$

Description:

A blank = Absorbance of DPPH solution in ethanol p.a.

A sample = Absorbance of test sample solution + DPPH solution in ethanol p.a.

A value of 0% means that it has no antiradical or antioxidant activity, while a value of 100% means total suppression and the test needs to be continued by diluting the test solution to see the concentration limit of its activity(Artikel et al., 2016).

Determination of IC_{50} Value

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The IC_{50} value is a number that indicates the concentration of the test sample that provides 50% suppression of DPPH free radicals (able to inhibit or reduce the oxidation process by 50%). Determination of the IC_{50} value is done by making a linear curve between the concentration of the test solution and the percent inhibition, namely by entering the concentration value of the test solution (ppm) as the X axis and the percent inhibition value (%) as the Y axis into the linear line equation. Specifically, a compound is said to be a very strong antioxidant if the IC_{50} value is less than 50 ppm, strong for IC_{50} values of 50-100 ppm, while if IC_{50} is 100-150 ppm and weak if IC_{50} is 151-200 ppm (Gustaman et al., 2022).

Data Analysis

The physical quality test data and IC50 value data of effervescent granules obtained were analyzed descriptively, namely by comparing the results obtained in the study with the physical quality standards of effervescent granules and antioxidant activity determination standards. Descriptive analysis is the most basic analysis to describe the general state of the data. Data on the IC50 value of effervescent granules obtained were also analyzed using the Analysis of Variance (ANOVA) test at a significant level of 95% and continued with the Honest Real Difference (HRD) test.

RESULT AND DISCUSSION

Results of Physical Quality Testing of Effervescent Granules of Red Dragon Fruit Peel Extract

Organoleptic Test

An organoleptic test was conducted by observing the color, odor, and taste of effervescent granule preparation of red dragon fruit peel extract. The results of organoleptic observations of effervescent granules can be seen in Table 2.

Table 2 Organoleptic Test Results of Effervescent Granules

Formula	Observation		
	Repeat I	Repeat II	Repeat III
1	White color, almost odorless, slightly sour taste	White color, almost odorless, slightly sour taste	White color, almost odorless, slightly sour taste
2	Light pink color, weak extract characteristic odor, weak sour taste	Light pink color, weak extract characteristic odor, weak sour taste	Light pink color, weak extract characteristic odor, weak sour taste
3	Pink color, characteristic odor of medium extract, medium sour taste	Pink color, characteristic odor of medium extract, medium sour taste	Pink color, characteristic odor of medium extract, medium sour taste
4	Dark pink color, strong extract characteristic odor, sour taste	Dark pink color, strong extract characteristic odor, sour taste	Dark pink color, strong extract characteristic odor, sour taste

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Physical quality testing of effervescent granule preparations is carried out to determine whether the effervescent granules made meet the physical quality requirements. Physical quality tests carried out include organoleptic tests, water content, flow speed, angle of repose, and dissolving time. Organoleptic testing includes odor, color, and taste tests. According to (9) in organoleptic testing, the results obtained should be the same as possible between one. Based on the test results, it is known that the effervescent granules produced have a uniform odor, color, and taste for each formula (Table 2).

Moisture content test

The results of the calculation of the % water content of effervescent granules of red dragon fruit peel extract can be seen in Table 3.

Table 3 Test results of effervescent granule Moisture content

Formula	granule Moisture content (%)			Average (%)
	Repeat I	Repeat II	Repeat III	
1	2.577	2.623	3.040	2.747
2	6.217	6.320	4.897	5.811
3	6.437	6.517	5.730	6.228
4	6.013	5.853	5.707	5.724

The results of the Moisture content test were obtained by calculating the difference in weight before and after drying. According to (Oriana Jawa La et al., 2020)low water content is good for storing preparations for a longer period, while high water content is a good medium for the growth of microorganisms, where microorganisms can grow well with water content above 10%. Based on the test results of effervescent granule water content, all formulas meet the requirements which are below 10% (Table 3).

Flow Speed and angle of repose Test

The test results of flow velocity and angle of repose of effervescent granules of red dragon fruit peel extract can be seen in Table 4 and Table 5.

Table 4 Effervescent Granule Flow Speed Test Results

Formula	Flow Speed (g/s)			Average (g/s)
	Repeat I	Repeat II	Repeat III	
1	4.598	4.444	4.494	4.512
2	4.494	4.545	4.444	4.494
3	4.124	4.384	4.255	4.254
4	3.922	4.000	4.082	4.001

Table 5 Test Results of angle of repose of Effervescent Granule

Formula	Angle of Repose			Average
	Repeat I	Repeat II	Repeat III	
1	28.058^0	28.058^0	26.565^0	27.560^0
2	26.565^0	28.058^0	26.565^0	27.063^0
3	28.058^0	26.565^0	28.058^0	27.560^0
4	28.058^0	28.058^0	28.058^0	28.058^0

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The flow speed and angle of repose tests were carried out by observing the time required for the effervescent granule to form a cone pile the flow speed, and the angle of the cone formed indicate the angle of repose. According to (Ika Pratiwi et al., 2022), the granule flow speed is said to be good if 4-10 g of granule can flow within 1 second, and the angle of repose is good if the angle of repose is between 25-300. Based on the results of the effervescent granule flow velocity test, all formula obtained are at a value between 4-10 g/s (Table 4), this shows that the effervescent granules produced have good flow properties. In the test of angle of repose obtained, the angle of repose of effervescent granules of all formulas is between 20-300 (Table 5), this shows that effervescent granules of all formulas have a good angle of repose.

Dissolving Time Test

The results of the effervescent granule dissolution time test of red dragon fruit peel extract can be seen in Table 6.

Table 6 Effervescent Granule Dissolving Time Test Results

Formula	Dissolving Time (Minute)			Average (Minute)
	Repeat I	Repeat II	Repeat III	
1	1.14	1.19	1.16	1.16
2	1.26	1.24	1.27	1.26
3	1.53	1.54	1.49	1.52
4	2.00	1.96	1.98	1.98

The dissolving time test was conducted with. According to (Hendra et al., 2020), a good effervescent granule dissolving time ranges between 1-2 minutes. Based on the results of the dissolving time test, it was found that the dissolving time of effervescent granules of all formulas met the requirements of dissolving time, which ranged from 1-2 minutes (Table 6).

Results of Antioxidant Activity Testing of Red Dragon Fruit Peel Extract Effervescent Granule

IC_{50} measurement results of effervescent granules of red dragon fruit peel extract can be seen in Table 7.

Table 7 Results of IC_{50} Value of Effervescent Granules

Formula	IC_{50} Value (ppm)			Average (ppm)
	Repeat I	Repeat II	Repeat III	
1	19.141	19.287	19.25	19.226
2	10.088	10.261	10.119	10.156
3	7.338	7.363	7.436	7.379
4	6.624	6.503	6.959	6.695

Antioxidant activity testing was conducted using the DPPH free radical inhibition method. This method is a test to determine the antioxidant activity in the sample to be tested by looking at its ability to counteract DPPH free radicals (1,1-diphenyl-2-picrylhydrazyl). Determination of antioxidant activity is expressed by IC_{50} value. The IC_{50} value is a number that shows the concentration of the test sample (ppm) that gives 50% DPPH silencing (able to inhibit or reduce the oxidation process by 50%). Based on the measurement

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results, the absorbance value decreases as the concentration of the test solution increases, but the % inhibition increases which causes the IC_{50} value to be smaller. According to (Manihuruk et al., 2017), a compound is said to have very strong antioxidant activity if the IC_{50} value is less than 50 ppm, a strong category for IC_{50} is 50-100 ppm, a medium category if IC_{50} is 100-150 ppm, and a weak category if IC_{50} is 151-200 ppm. The effervescent granules of red dragon fruit peel extract in all formulas have an average IC_{50} value of less than 50 ppm (Table 7), this shows that the effervescent granules obtained have very strong antioxidant activity. The antioxidant activity value of F1 (negative control) is also very strong due to the presence of citric acid in the effervescent granule-making material, where citric acid is one of the chelator class antioxidant compounds (Forestryana et al., 2020).

Data analysis of the IC_{50} value of effervescent granules using Analysis of Variance (ANOVA) at a significant level of 95%, results showed that there were significant differences between the four effervescent granule formulas. Based on the Coefficient of Variance (COV) value obtained, which is 0.31%, further tests were carried out using the Honest Real Difference (BNJ) test, the results showed that the antioxidant activity of effervescent granules of red dragon fruit peel extract F1, F2, F3, and F4 was significantly different. Based on the results of the analysis conducted, it can be seen that the variation in the concentration of red dragon fruit peel extract used has a significant effect on the antioxidant activity of the effervescent granules produced.

CONCLUSION

Based on the research results obtained, it can be concluded that red dragon fruit peel extract can be formulated into effervescent granules. The observation shows that effervescent granules of all concentrations have a physical quality that meets the requirements and have very strong antioxidant activity with IC_{50} values of less than 50 ppm.

ACKNOWLEDGMENTS

We would like to express our gratitude to Yayasan Pelita Mas Palu for the funding to carry out the research, and our gratitude also goes to the Institution of Pelita Mas College of Pharmacy Palu for facilitating us to carrying out the research.

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